Increased Plasma Incretin Concentrations Identifies a Subset of Patients with Persistent Congenital Hyperinsulinism without K_{ATP} Channel Gene Defects

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Congenital hyperinsulinism causes profound hypoglycemia, which may persist or resolve spontaneously. Among 13 children with congenital hyperinsulinism, elevated incretin hormone concentrations were detected in 2 with atypical, persistent disease. We suggest that incretin biomarkers may identify these patients, and that elevated hormone levels may contribute to their pathophysiology. *(J Pediatr 2015;166:191-4).*

Congenital hyperinsulinism (CHI), characterized by inappropriate release of insulin from pancreatic β cells, is associated with brain injury due to hypoglycemia.[1-3] The severity of hyperinsulinism varies and may be transient or persistent. More than 70% of all patients with CHI currently have no identified genetic basis.[4-6] The most common causes of persistent CHI are mutations in the *ABCC8* and *KCNJ11* genes, which cause focal (CHI-F) or diffuse (CHI-D) histological variants of CHI.[1,2] A recently described histopathological variant of CHI, termed atypical CHI (CHI-A), accounts for 10% of patients undergoing pancreatectomy for treatment of CHI.[7] Diagnosing CHI-A is difficult because no associated mutations have been reported, affected patients are variably responsive to medications, and imaging with fluorine-18-labeled L-dihydroxyphenylalanine positron emission tomography and computed tomography, which differentiates between CHI-F and CHI-D, is unable to identify CHI-A.[7]

The incretin hormones glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) are secreted from enteroendocrine cells and exert a substantial regulatory influence on insulin secretion.[8] The aim of the present study was to compare fasting and postprandial plasma GLP-1 and GIP concentrations among patients with persistent forms of CHI (CHI-F, CHI-D, and CHI-A) and those patients with transient disease. Our results suggest a positive association between an elevated GLP-1 (7-36):GIP and CHI-A, which should be investigated as a potential diagnostic biomarker for CHI-A.

Methods

Thirteen patients with CHI were recruited with local Ethical Board approval and parental consent at a UK National Referral Centre for CHI. The **Table** summarizes the patients' clinical profiles, with the classification of CHI based on established diagnostic criteria.[1,2,4,9] Sequence analysis of the exons and intron/exon boundaries of the *ABCC8* and *KCNJ11* genes was performed on all patients using genomic DNA extracted from peripheral blood leukocytes. *ABCC8* analysis included screening for the recently reported deep intronic cryptic splicing mutation.[10] No further testing was performed in the patients with transient CHI. In the patients with atypical disease, Sanger sequencing of the exons and intron/exon boundaries of the *HADH*, *GCK*, and *HNF4A* genes was performed. *HNF4A* analysis included the coding exons 1d-10 and the P2 pancreatic promoter, and *HADH* analysis included screening for the deep intronic splicing mutation.[10]

For analysis of plasma peptides, patients fasted for 4 hours before blood sample collection and more bloods samples were collected 20 minutes (30 minutes for patients subjected to an oral glucose tolerance test) after the start of feeding. Formula feeds (**Table**), obtained from Nutricia (Trowbridge, United Kingdom), were given at a rate of 20 mL/kg body weight. Two patients (2 and 13) were subjected to standard oral glucose tolerance test.

All samples were centrifuged at 1300 × *g* for 10 minutes at 4°C, after which the plasma was removed and stored at

CHI	Congenital hyperinsulinism
CHI-A	Atypical congenital hyperinsulinism
CHI-D	Diffuse congenital hyperinsulinism
CHI-F	Focal congenital hyperinsulinism
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1

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Table. Clinical characteristics of the CHI patient cohort

Patient	Classification of CHI	Age at diagnosis	Age at sample collection, mo	Birth weight, kg	Gestation, wk	Genetic cause	Medical treatment	Surgical treatment	Outcome	Feed
1	Transient	6 d	1	2.08	38	Unknown	Diazoxide	No	Normoglycemic	Breast milk
2	Transient	1 y	190	3.45	40 + 2	Unknown	Diazoxide	No	Normoglycemic	OGTT
3	Transient	4 d	16	2.86	40	Unknown	Diazoxide	No	Normoglycemic	Nutrini Peptisorb
4	Transient	2 d	24	3.5	40	Unknown	Diazoxide	No	Normoglycemic	Polycal
5	Transient	1 d	1	2.49	40	Unknown	Diazoxide	No	Normoglycemic	Cow & Gate/ Polycal
6	Diffuse	2 d	27	4.4	40	*ABCC8* Comp hetero	Octreotide	No	Continuous therapy	Nutrini Peptisorb
7	Diffuse	3 d	26	3.25	40	*ABCC8* Maternal	Diazoxide	No	Continuous therapy	Nutrini Peptisorb
8	Diffuse	4 d	51	3.4	42	*KCNJ11* Paternal	Diazoxide/ octreotide	No	Continuous therapy	Nutrini Peptisorb
9	Diffuse	6 d	33	4.73	40	*ABCC8* Homozygous	Diazoxide/ octreotide	Near-total pancreatectomy	Cured	Whole milk
10	Focal	3 mo	49	3.62	40	*ABCC8* Paternal	Diazoxide	Subtotal pancreatectomy	Cured	Whole milk
11	Focal	7 d	48	4.9	38 + 5	*ABCC8* Paternal	Diazoxide/ octreotide	Subtotal pancreatectomy	Cured	Infantrini
12	Atypical	2 y, 7 mo	36	2.72	34	Unknown	Diazoxide/ octreotide	Near-total pancreatectomy	Cured	Nutrini Peptisorb
13	Atypical	21 mo	37	3.4	40	Unknown	Diazoxide	No	Continuous therapy	OGTT

Comp hetero, compound heterozygous; *OGTT*, oral glucose tolerance test.

All patients were treated for hypoglycemia and classified as transient, diffuse, focal, or atypical based on clinical characteristics, genotyping, fluorine-18 labeled L-dihydroxyphenylalanine positron emission tomography and computed tomography diagnosis or pancreatic histology after surgery. Four patients with persistent disease (patients 9-12) underwent surgery to alleviate hyperinsulinism; samples for this study were obtained after recovery from surgery. Patients 1-5 and 8-11 were sampled in the absence of drug treatment. For transient patients, disease resolution occurred by follow-up visits at age 8 months (patient 1), 5 years (patient 2), 9 months (patient 3), 15 months (patient 4), and 5 months (patient 5). Four patients with persistent disease are currently receiving medical interventions (patients 6, 7, 8, and 13) and were sampled during ongoing treatment.

−80°C before analysis. Plasma GLP-1 (7-36) and total GIP concentrations were assessed in triplicate by enzyme-linked immunosorbent assay (ALPCO, Salem, New Hampshire and EMD Millipore, Billerica, Massachusetts, respectively). Incretin hormone concentrations and ratios were analyzed for differences between all patient groups. Statistical significance was determined by 1-way ANOVA and the Tukey post hoc test where appropriate, with a P value <.05 considered significant. All data are presented as mean ± SEM.

Results

All patients were screened for mutations in *ABCC8*/*KCNJ11* (Table). Two patients (12 and 13) had late-onset presentation of persistent CHI, with no characteristic triggers for hypoglycemic events, and both were genotype-negative for defects in *ABCC8*, *KCNJ11*, *HADH*, *GCK*, and *HNF4A*. After decline and eventual failure of responses to medical therapy, patient 12 required a 95% pancreatectomy. Examination of the resected pancreas revealed a heterogeneous pattern of pancreatic histopathology and abnormal expression of hexokinase 1, suggestive of CHI-A based on published criteria.[7,11]

In all patients, postprandial incretin concentrations increased above basal levels, with no differences between the patients with CHI-F and those with CHI-D when analyzed separately (Figure). The average basal GLP-1 (7-36) and GIP concentrations were not significantly different across the patient groups (transient CHI vs persistent CHI-F/CHI-D and CHI-A). On average, postprandial GLP-1 (7-36) concentrations increased to 3.9 ± 0.9 pmol/L in the patients with transient CHI (n = 4) and to 4.3 ± 1.0 pmol/L in those with CHI-F/CHI-D (n = 6), and was markedly higher in the patients with CHI-A, measured at 82.2 pmol/L in patient 12 and 16.5 pmol/L in patient 13 ($P < .05$; Figure, A). These 2 patients with atypical disease also exhibited greater fold changes in postprandial GIP concentrations, increasing by 14-fold, compared with 8-fold in patients with transient CHI and 7-fold in those with CHI-F/CHI-D (Figure, B).

The postprandial GLP-1 (7-36):GIP was similar in patients with transient CHI and those with CHI-F/CHI-D (0.1 ± 0.03 [n = 4, patients 1-4] vs 0.13 ± 0.03 [n = 6, patients 6-11]), but was markedly elevated in patients with CHI-A (0.52 ± 0.06, $P < .001$; n = 2, patients 12 and 13) (Figure, C).

Discussion

Incretin hormones have potent regulatory effects on hormone secretion, and inappropriately increased GLP-1 or GIP secretion after gastric surgery has been linked to hyperinsulinemic hypoglycemia.[12,13] In the present study, 2 patients with CHI-A had strikingly higher postprandial GLP-1 (7-36):GIP compared with patients with CHI-F, CHI-D, or transient CHI (Figure).

In patients with CHI-A, the genetic basis of disease is unknown and may vary, although heterogeneous expression of hexokinase 1 in β cells is associated with inappropriate insulin release in atypical patients requiring pancreatectomy.[11]

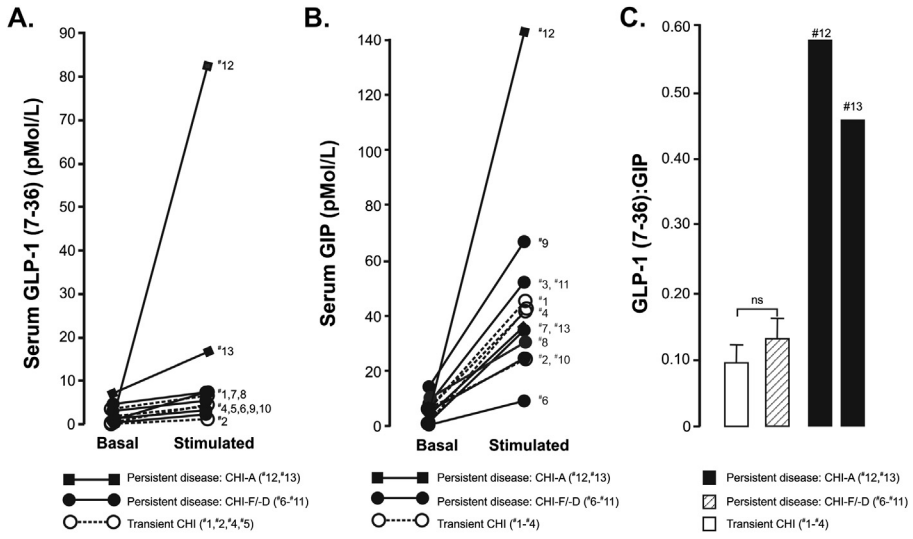

Figure. **A** and **B,** Basal and postprandial ("stimulated") concentrations of GLP-1 (7-36) and GIP in patients with CHI. **C,** GLP-1 (7-36):GIP in the 3 patient groups. The integrated incretin ratio values showed no differences in the transient and CHI-F/CHI-D patient groups, but was significantly larger in patients with CHI-A ($P < .001$).

We found a similar profile of hexokinase 1 expression in β cells in the tissue of patient 12, but could not evaluate this in patient 13, who was stable on medical therapy. Interestingly, studies on rat insulinoma cell lines have demonstrated up-regulation of hexokinase 1 gene expression in response to GLP-1 and GIP in vitro, suggesting a possible link between incretin pathology and CHI-A.[14,15]

Despite variable incretin secretion stimuli in the patients in this study and ongoing medical treatment that may have minimized observed differences, both patients with CHI-A had a markedly elevated postprandial GLP-1 (7-36):GIP compared with the other patient groups. Our preliminary findings suggest an alternative cause of hyperinsulinemic hypoglycemia independent of K_{ATP} channel–driven mechanisms that may affect β cells directly or may increase insulin secretion via enhanced incretin action. We speculate that both of our patients with CHI-A possibly would have benefited from the GLP-1 receptor antagonist–based therapy described by Calabria et al,[16] which in 1 patient might have obviated the need for near-total pancreatectomy.

In summary, CHI-A currently represents a subgroup of patients with persistent and medically unresponsive hypoglycemia that cannot be detected by imaging techniques and for which there is no genetic or endocrine biomarker. Based on our observations, we suggest that investigation of plasma incretin profiles in patients with CHI may identify this group of patients to be followed up in multicenter studies. ■

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